The Self Marker Concept as Applied to the Rh Blood Group System

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A PERTINENT FEATURE OF ANTIBODY FORMATION is the fact that an organism does not produce antibodies against its own body constituents—materials which will elicit antibody formation when injected into other organisms. To account for this fact, the "self marker" concept was advanced by Burnet and Fenner (1948, 1949). The essence of this concept is that as a developing fetus is being exposed to its own antigenic substances, the fetus acquires the ability to distinguish these as "self" in contrast with foreign antigenic substances as "not-self."

Immunization of a mother by a fetal red blood cell antigen other than A or B was reported by Levine and Stetson in 1939. Subsequent investigations by Levine, Burnham, Katzin and Vogel (1941) showed that the blood factor involved was either identical with or related to the Rh antigen described by Landsteiner and Wiener in 1940. Although extensive studies have been carried out since 1939 on the Rh system, no satisfactory explanation has been given for the fact that immunization against the Rh antigen occurs in only a small proportion of the Rh negative women who might be expected to exhibit this reaction. A commonly ascribed reason for the failure of Rh negative women to produce antibodies against the Rh antigen of their Rh positive fetuses is the impermeability of the placental membrane to the fetal erythrocytes. However, this impermeability is not perfect in all instances and Cohen, Zuelzer and Evans (1960) presented evidence of fetal erythrocytes in the maternal circulation.

Another factor which must be considered regarding this problem was first pointed out by Levine (1943). He observed that mothers of children with erythroblastotic disease due to anti-Rh were more often compatibly mated in the ABO system than were unselected women. This has been confirmed by others. Thus some Rh negative women who are incompatibly mated in the ABO system fail to produce Rh antibodies because the fetal erythrocytes which reach the maternal circulation are destroyed by their naturally occurring agglutinins. These erythrocytes are thus unable to serve as an adequate source of the Rh antigen.

A third hypothesis to explain the failure of many Rh negative women to produce the expected anti-Rh antibodies involves the "self marker" concept. If an Rh negative woman was exposed to Rh positive erythrocytes from her Rh positive mother while a fetus, she might develop a tolerance to this antigen because it would act as a "self" substance. Upon subsequent contact with the antigen from her own fetus, she would then fail to develop antibodies. This hypothesis

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is suggested by an observation of Owen, Wood, Foord, Sturgeon and Baldwin (1954). They classified Rh negative women as being relatively tolerant if they failed to develop the Rh antibody within three Rh positive pregnancies, and as being relatively intolerant if they developed the Rh antibody during or before the third Rh positive pregnancy. Of 34 cases of relatively tolerant women, 25 had Rh positive mothers and only 9 had Rh negative mothers. Of 17 cases of relatively intolerant women, only 4 had Rh positive mothers while 13 had Rh negative mothers. These data suggest a strong relationship between tolerance (failure to produce Rh antibodies) and the Rh positive blood group of the mother of the Rh negative woman.

All data do not agree with those of Owen *et al.* (1954). Booth, Dunsford, Grant and Murray (1953) investigated the Rh type of the maternal grandmothers of 113 children suffering from hemolytic disease due to Rh sensitization. They found 46 grandmothers to be Rh negative, which is the expected number to be found among 113 randomly picked mothers of ordinary Rh negative persons (expected *Dd*: 113 x 0.5897; expected *dd*: 113 x 0.4103). Ward, Walsh and Kooptzoff (1957) reported the Rh status of mothers of 173 Rh negative women who gave birth to children afflicted with hemolytic disease. Their conclusions were similar to those of Booth *et al.* in that they found no correlation between the maternal grandmother's Rh type and the infant's likelihood of developing hemolytic disease.

The investigations here reported were undertaken to obtain additional data concerning the "self marker" concept as applied to the Rh_0 (D) blood group system.

MATERIAL AND METHODS

The Rh negative women were ascertained through the blood bank records of the Latter-day Saints Hospital in Salt Lake City, Utah. It is the practice of this hospital to take a cord blood sample at the time of parturition of each Rh negative woman regardless of her past history and without regard to the blood type of the husband. The cord blood sample is sent to the hospital blood bank and the ABO and Rh type of the child's blood is determined. In addition, an antiglobulin determination is carried out by the direct Coombs test. A positive Coombs test indicates that the mother had produced antibodies against the child's erythrocyte antigens and that these had crossed the placenta and coated the infant's red blood cells.

The medical records of these women were obtained through the cooperation of the hospital. The records were examined to determine the total number and the Rh blood type of previous children. To make this study comparable to that of Owen *et al.* (1954), only those women who had given birth to a total of three or more (including the index birth) Rh positive children were considered. If the woman had terminated all her pregnancies at the Latter-day Saints Hospital, the essential information on the children was readily available. If some of her pregnancies were terminated elsewhere, the Rh type of her children would not be known unless the attending physician had made this a part of her record.

The women with these characteristics were interviewed by telephone and in each case her mother's name, address and telephone number were obtained. If the children's Rh types were unknown, arrangements were made to type the children. Where possible, the husband's blood type was obtained from records or he was typed later. The woman's mother was then interviewed by telephone and questioned regarding her ABO and Rh types. If she had a record of these from having been a blood donor or recipient, she was not retyped. Otherwise, permission was obtained and arrangements were made to type her blood.

The original contact with each family was through the Rh negative woman. Because these women understood the nature of the Rh problem, they were eager to cooperate and furnish information even though the majority of them had not had erythroblastotic babies. The cooperation of the family as a unit was virtually 100 per cent. Only one grandmother refused to be typed.

The blood sample for typing was obtained by pricking the finger with a sterile, disposable lancet. Three to four drops of blood were collected in a small test tube containing one to two ml. of isotonic saline solution. Before typing, the cells were washed three times in isotonic saline. Typing sera were obtained from the Holy Cross Hospital Research Foundation, Salt Lake City, Utah. All agglutination tests were carried out in standard Kahn tubes at room temperature and the directions for the use of the sera were followed explicitly. All typings were done on the day blood samples were collected.

For Rh typing, 3 drops of a 2 per cent suspension of the cells in isotonic saline were placed in a Kahn tube containing one drop of human anti-Rh_0 (anti-D) serum plus three drops of 10 per cent polyvinylpyrrolidone. This latter substance is prepared by the Holy Cross Hospital Research Foundation specifically for a rapid Rh tube test. When the Rh typing with anti Rh_0 (anti-D) serum gave a negative result, an indirect antiglobulin test was made to determine whether or not the D^u variant of the antigen was present.

The Rh negative women were then classified as relatively tolerant if they failed to produce the Rh antibody on or before their third Rh positive pregnancy, and were classified as relatively intolerant if they developed anti-Rh antibody on or before the third Rh positive pregnancy. This was also the classification used by Owen *et al.* (1954). However, in this study the method of detection of the production of the antibody was different from that used by Owen *et al.* The women classified as relatively tolerant had shown a negative Coombs test of the cord blood of the index birth and presumably, therefore, had shown negative Coombs tests on prior Rh positive births. Those classified as being relatively intolerant had shown a positive Coombs test on the cord blood of the index birth and in addition had shown a positive Coombs test on or before her third Rh positive birth as determined by the Latter-day Saints Hospital or other medical records. Cases were eliminated when the woman in question had a history of blood transfusion.

The study began with 8,500 names of Rh negative women who had terminated their pregnancies at the Latter-day Saints Hospital during the period from March, 1954, to March, 1960. After the elimination of all cases which failed to meet the criteria given above, a total of 210 cases remained. These consisted of family units in which the Rh blood type of the maternal grandmother, the Rh negative mother and three or more Rh positive children were known. Of these family units, the data for 158 include the ABO types of the woman and husband as well as the Rh type. These data were used to study the effect of ABO mating types on Rh sensitization.

RESULTS

The thesis of Owen and associates was that Rh negative women who had Rh positive mothers would receive Rh antigens from the mothers during prenatal development. As a result, through the application of the self marker concept, the antibody producing cells of these Rh negative women would recognize the Rh antigen as "self" and therefore have a reduced ability to develop antibodies against them. These women would tend to fall into Owen's classification of tolerant. Rh negative women who had Rh negative mothers could not experience such a prenatal exposure to the antigen and therefore would be capable of developing Rh antibodies. These women would tend to fall into the intolerant group. The data from this study on the relationship between tolerance and intolerance of the Rh negative women and the Rh blood type of their mothers are presented in table 1.

TABLE 1. Rh TYPES OF MOTHERS OF Rh NEGATIVE WOMEN

Classification of Rh negative daughters	Mother's Rh Type		Total
	Rh +	Rh −	
Tolerant (non-sensitized)	84	59	143
Intolerant (sensitized)	37	30	67
Total	121	89	210

The table shows that there were 143 Rh negative women classified as tolerant. If the self marker concept should apply, then a larger proportion of these Rh negative women should be the daughters of Rh positive mothers than would be expected from a normal, unselected population. Using the gene frequency figure of 0.4103 for the *d* gene as given by Race and Sanger (1958, p. 132) for an English population, the expected number of Rh positive mothers of 143 ordinary Rh negative persons can be calculated. This number is 84.3, as compared to the observed number of 84. The deviation observed is not significant. Data obtained from the Regional Red Cross Blood Bank in Salt Lake City indicate that the frequency of the *d* gene in the Salt Lake region does not differ significantly from the English population.

Table 1 further shows that there were 67 Rh negative women classified as intolerant. Of these, 30 had Rh negative mothers and 37 had Rh positive mothers. The expected number of Rh positive mothers of 67 normal Rh negative persons is 39.5. The chi-square value of the deviation is 0.38, corresponding to P value between 0.70 and 0.50.

It is evident from the data that the phenomenon of tolerance and intolerance among the Rh negative women who have had an opportunity to become sensitized to the Rh antigen bears no relationship to the Rh blood type of their mothers and that an explanation of the tolerance phenomenon must be sought elsewhere.

Since the observation by Levine (1943) that ABO incompatible matings tend to protect the Rh negative mothers from becoming immunized to the Rh antigen of their Rh positive fetuses, numerous investigations have confirmed this phenomenon. Furthermore, Levine (1958, 1961) has suggested that the data of Owen *et al.*, can be explained on the basis of the ABO mating types quite as well as on the basis of the applicability of the self marker concept. Therefore, it becomes necessary to examine the data obtained in this study from the standpoint of the ABO mating type.

There were 210 Rh negative women who had an opportunity to become sensitized to the Rh antigen. The ABO blood types of both the husband and wife were known for 158 of these and the ABO compatible or incompatible mating types were determined. A compatible mating in the ABO system is one in which the wife's serum fails to react with the husband's erythrocytes. An incompatible mating is one in which the wife's serum will agglutinate these erythrocytes. When the husband's blood type is compatible, all offspring will

have a blood type compatible with the wife's type. However, when the husband's blood type is incompatible, some offspring may have blood compatible with the wife's blood while others may be incompatible. It is the latter group of offspring which bear on the problem of the failure of Rh negative women to become sensitized to the Rh antigen. It would have been desirable to obtain the ABO blood type of all the Rh positive offspring of the Rh negative women used in the study. This was obtained for some but the data are incomplete. By using the mating type rather than the offspring's blood type, the quantity of the data is increased and is comparable in size with the data of other studies.

The Rh negative women of both types can be classified as to their mating types in the ABO system. Table 2 shows this relationship. Ninety-seven of the

TABLE 2. EFFECT OF ABO MATING TYPE ON Rh SENSITIZATION

ABO Mating	Rh Sensitization		
	Sensitized	Non-sensitized	Total
Compatible	44	53	97
Incompatible	12	49	61
Total	56	102	158

158 women were compatibly mated. Of these, 44 were sensitized and 53 non-sensitized to the Rh antigen. There were 61 women who were incompatibly mated. Of these, 12 were sensitized and 49 were non-sensitized. A 2 x 2 contingency table to test the relationship of ABO incompatibility to Rh sensitization gave a chi-square value of 11.6, and a P value less than 0.001. Thus, this study confirms Levine's (1943, 1958) findings, although they were taken from a group of Rh negative women selected on the basis of having had an opportunity for Rh sensitization.

To determine whether this sample, so selected, is a representative sample, the figures in table 2 were compared with Levine's estimates for samples picked at random from a normal population as regards ABO mating type when the husband is Rh positive and the wife is Rh negative. According to Levine's estimate (1958), there should be approximately 65 per cent compatible matings and 35 per cent incompatible matings in a sample picked at random from a normal population without regard to Rh sensitization. There are 61 per cent compatible matings and 39 per cent incompatible matings in the sample represented in table 2. The difference is not significant when Levine's values are used as the expected values ($\chi^2 = 1.00$; $P > 0.30$). Furthermore, Levine (1958) estimated that there should be approximately 83 per cent ABO compatible matings and 17 per cent ABO incompatible matings in a group of women who are sensitized to the Rh antigen. Table 2 shows that there are 56 women sensitized to the Rh antigen; of these 44 (79 per cent) are compatibly mated and 12 (21 per cent) are incompatibly mated. The difference is not significant ($\chi^2 = 0.70$; $P > 0.30$). The data, therefore, show that this group of 158 Rh negative women, all of whom had an opportunity to become sensitized, represents an unbiased sample as regards the frequency of compatible and incompatible matings in the ABO blood group.

TABLE 3. Rh TYPES OF MOTHERS OF Rh NEGATIVE WOMEN COMPATIBLY MATED IN THE ABO SYSTEM

Classification of Rh negative daughters	Mother's Rh type +	−	Total
Tolerant (non-sensitized)	29	24	53
Intolerant (sensitized)	20	24	44
Total	49	28	97

The data from this study, as presented in table 3, permit a re-examination of the applicability of the self marker concept to Rh sensitization uncomplicated by the ABO mating type. If an increase in non-sensitization can be demonstrated in this group, it might be presumed to be the result of the Rh type of the women's mothers.

The data were analyzed by the use of a 2 x 2 contingency table, testing the hypothesis that the mothers of both the sensitized and non-sensitized women were drawn from the same population. A chi-square of 0.829 was obtained, which gives a P value greater than 0.30. Admittedly, the sample is relatively small, but in this group of Rh negative women in which the mating type in the ABO system is not a factor, there is no statistical evidence that the Rh type of the mother influences the failure of daughter to become sensitized to the Rh antigen.

DISCUSSION

Fudenberg, Kunkel and Franklin (1958) demonstrated that the Rh antibody specific for the Rh_0 (D) is of two types: (1) The so-called complete antibody which agglutinates Rh_0 (D) cells in saline, has a molecular weight of approximately 1,000,000 and a sedimentation constant of 18 Svedberg units. (2) The so-called incomplete antibody, which requires a protein medium for the agglutination of Rh positive cells, has a molecular weight of approximately 160,000 and a sedimentation constant of 6.5 Svedberg units. The complete antibody will not pass the placental membrane while the incomplete antibody will readily cross this barrier. If this is generally true, then the Rh antibody which is important in causing hemolytic disease in the newborn is the incomplete antibody. This is the antibody tested for in this study.

In this investigation, the method used to classify Rh negative women as relatively tolerant or intolerant is different from that used by Owen *et al.*, (1954). They tested for the presence of Rh antibodies during the course of pregnancy in the maternal circulation. Their methods allowed them to detect both complete and incomplete antibodies. In this study the direct Coombs test, which detects only the incomplete antibody, was applied to the cord blood of the newborn. Consequently, any complete antibody which might have been present in the maternal circulation was not detected. Moreover, the method for the detection of Rh antibodies in this study is different than the one used in the

studies of Booth *et al.* (1953) and Ward, Walsh and Kooptzoff (1957). In these studies the production of Rh antibodies by Rh negative mothers was inferred from the fact that they had given birth to children diagnosed as erythroblastotic. All of these women would be intolerant according to the classification of Owen *et al.* (1954). In this study, the Rh antibodies were detected in the cord blood. Therefore many cases were included in which the mother produced antibodies but in which erythroblastosis did not develop in the Rh positive child. All of these women were classified as intolerant. If the antibodies were not detected, these women were classified as tolerant. Therefore, the method used for detection of Rh antibodies in this study is less sensitive than that of Owen *et al.*, but more sensitive than that of Booth *et al.* and Ward *et al.*

The data of Owen *et al.* (1954) suggest that the self marker concept may apply to human antibody formation in the Rh system, but as can be seen, contradictory evidence is available also. Levine (1958, 1961) suggested that the data of Owen *et al.* can be explained on the basis of the ABO mating type. In the same way, there are contradictory data regarding the concept as it applies to the ABO blood group system. Jakobowicz, Crawford, Graydon and Pinder (1958, 1959) tested army trainees (aged 18-21) who were blood group O for anti-A antibody titer before and after immunization with tetanus toxoid. They found that the anti-A antibody titer was significantly higher in those trainees with type O mothers than in those with type A mothers, suggesting that *in utero* exposure to the A antigen (if secretor) might be the cause of lower anti-A antibody titer. However, Tiilikainen, Lehtovaara and Eriksson (1959), Wicher and Wozniczko-Orlowska (1960) and Mayeda (unpublished data) found no evidence of this phenomenon.

If the self marker concept is to apply to the human blood group antigens, then it must be assumed that the placental membrane is permeable to the maternal erythrocytes, or to fragments of the maternal erythrocytes carrying the appropriate antigens. There is good evidence for the passage of the fetal erythrocytes into the maternal circulation, as shown by the sensitization of the Rh negative woman by her Rh positive fetus. But evidence for the passage of maternal erythrocytes into the fetal circulation is meager. The evidence for this passage has been obtained by injecting abnormal erythrocytes such as elliptocytes and sickle cells into the blood of pregnant women and recovering them in the cord blood at birth. (Hendenstedt and Naeslund, 1946; Mengert, Rights, Bates, Reid, Wolf, and Nabors, 1955). The small number of cases and the difficulty of a positive identification of the marker cells leave the passage of maternal erythrocytes through the placenta still in doubt. Results obtained using erythrocytes labeled with radioactive isotopes should be regarded with caution, as radioactive components disassociated from the erythrocytes might readily cross the placental membrane and enter the fetal circulation.

From consideration of the structure and physiology of the placenta, it is difficult to visualize how maternal erythrocytes might pass the placental membrane. The blood on the maternal side of the membrane flows through open intervillous spaces while that on the fetal side is contained within a closed system of vessels. Thus the maternal blood should have a lower hydrostatic pressure than

the fetal blood and any discontinuity of the placental membrane should result in a loss of fetal cells to the maternal system rather than the reverse.

Even if the placenta should prove to be permeable to maternal erythrocytes, there is evidence that the antigen must reach the fetus at a critical stage of development to produce marked tolerance. This was shown by studies in the chick (Simonsen, 1955) where maximum response was obtained when the antigen reached the embryo between the tenth and seventeenth days of incubation. If this should be true for human embryos, the maternal erythrocytes must enter the fetal system during a relatively limited but unknown period of time. Statistical studies such as this could not identify the relatively few cases in which this might occur and in which the self marker concept might apply. Certainly the data obtained in this study do not support the idea that any large proportion of Rh negative women who might be expected to develop antibodies, but do not do so, can be accounted for by the self marker mechanism.

SUMMARY

The essence of the self marker concept is that a fetus exposed to an antigen *in utero* will be unable or have reduced ability to produce antibodies against that antigen in later life, even though immunologically competent and capable of producing antibodies against other antigens. The correctness of this concept has been shown in certain experimental animals. Reports have been made of the applicability of this concept to human blood group antigens in the Rh and the ABO blood group systems, but contradictory evidence has also been reported.

In this study, 210 Rh negative women who had an opportunity to become sensitized to the Rh antigen by pregnancy were ascertained. These women had at least three Rh positive children, including the index birth. The Rh types of the mothers of these 210 Rh negative women were determined.

Analysis of the data shows no correlation between the mother's Rh type and her Rh negative daughter's failure to produce Rh antibodies. There was no clear, consistent tendency for tolerant daughters to be the offspring of Rh positive mothers and for intolerant daughters to be the offspring of Rh negative mothers, as would be expected if the self marker concept is applicable.

It has been demonstrated elsewhere that ABO incompatibility between a mother and her fetus tends to protect Rh negative women from becoming sensitized to the Rh antigen. Therefore, the question of ABO compatibility of the mating should be taken into consideration. Among the original 210 Rh negative women, a sub-group of 97 women with ABO compatible matings was identified. In this sub-group there was no relationship between the daughter's ability to produce Rh antibody and the mother's Rh blood type. Thus, when the complicating factor of ABO incompatibility is removed, the data also lead to the conclusion that the self marker concept does not apply to Rh sensitization.

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